Sustained hydrogen photoproduction by *Chlamydomonas reinhardtii* — Effects of culture parameters

S. Kosourov [a], A. Tsygankov [a,b], M.L. Ghirardi [a] and M. Seibert* [a]
[a] National Renewable Energy Laboratory, Golden CO 80401, USA
Fax: 303-384-6150; Mike_Seibert@nrel.gov
[b] Institute of Basic Biological Problems, Pushchino, Moscow Region, 142292, Russia

The green alga, *Chlamydomonas reinhardtii*, is capable of sustained H_2 photoproduction when grown under sulfur-deprived conditions [1,2]. This ability is the result of partial inactivation of photosynthetic O_2-evolution activity in response to sulfur deprivation, which results in the establishment of an anaerobic environment in the culture due to oxidative respiration under continuous illumination. Algae will not produce H_2 under aerobic conditions. After 10-15 hours of anaerobiosis, sulfur-deprived algal cells induce the reversible hydrogenase and start evolving H_2 in the light. Using a computer-monitored photobioreactor system, we investigated the behavior of sulfur-deprived algal cultures and found that:

(a) they transition through the following four consecutive phases: an aerobic phase, an O_2-consumption phase, an anaerobic phase and a H_2-production phase (Fig. 1);
(b) synchronization of cell division by light-and-dark cycles leads to earlier establishment of anaerobiosis in the cultures and to earlier onset of the H_2-production phase (Fig. 2);
(c) re-addition of small quantities of sulfate (12.5 – 50 ?M $MgSO_4$, final concentration) to the synchronized or unsynchronized cell suspensions can result in an initial increase in culture density, a higher specific initial rate of H_2 production, an increase in the length of the H_2-production phase, and an increase in the total H_2 output (Fig. 2); and
(d) increases in the culture optical density in the presence of 50 ?M sulfate result in decreases in the initial specific rates of H_2 production but in early starts of the H_2-production phase (Fig.3).

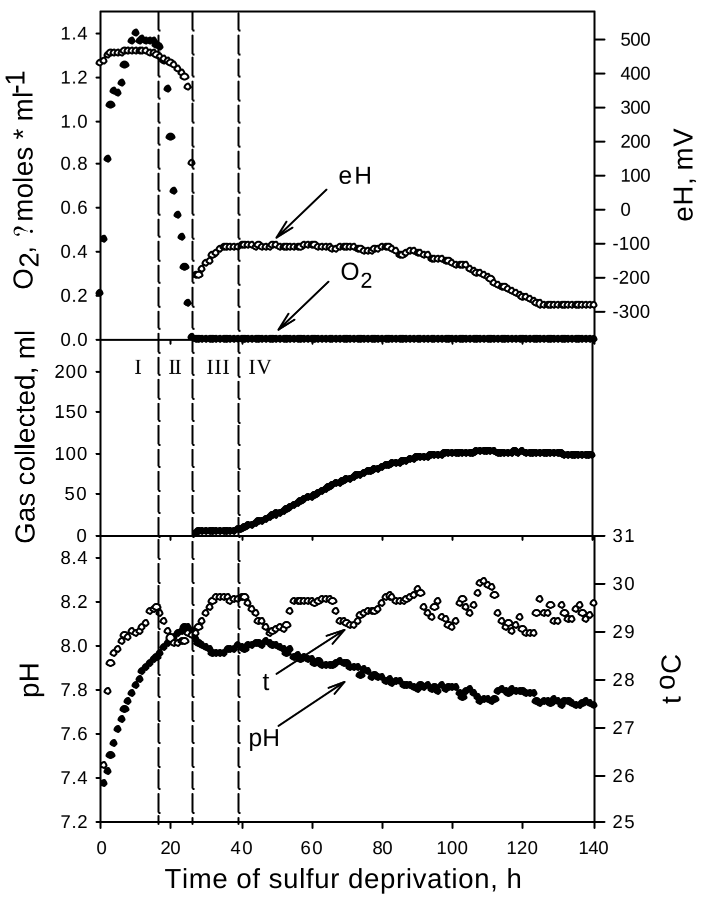

Figure 1. Changes in pO_2, eH, pH, temperature, and volume of gas collected during the incubation of synchronized cells under sulfur-deprived conditions. Four transition phases, indicated as I-IV, are identified.

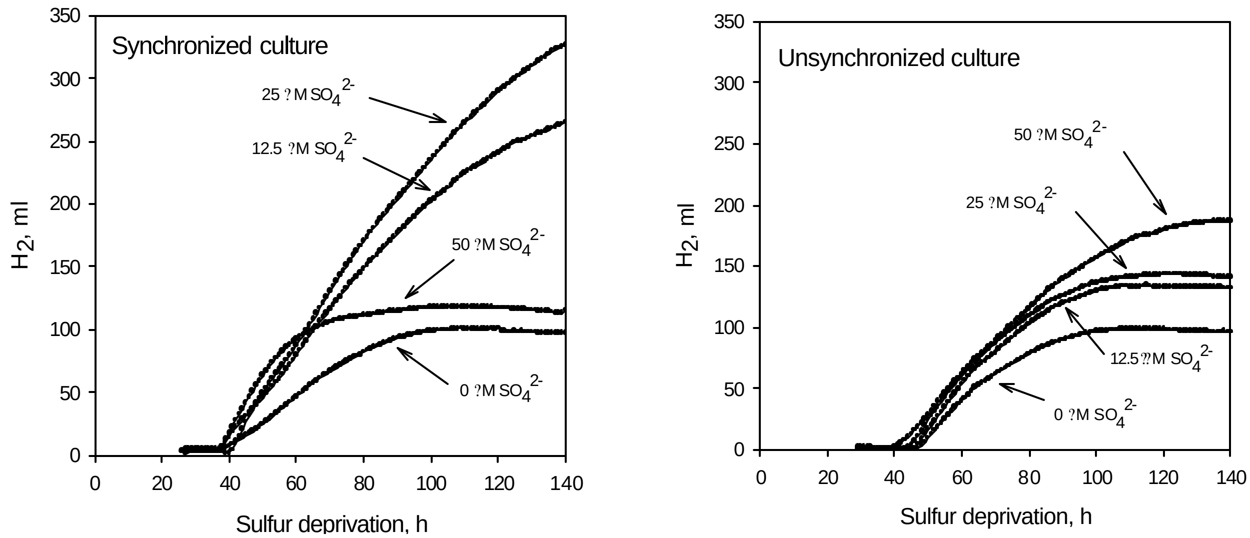

Figure 2. Effect of re-added sulfate at time = 0 on H_2 production in synchronized and unsynchronized sulfur-depleted cultures of *C. reinhardtii*.

We suggest that the effects of re-adding micromolar concentrations of sulfate on H_2 production are due to its influence on the residual O_2-evolution activity of photosystem II upon which most of the electrons for H_2 production depends [3]. We conclude that optimization of H_2 production in the system can be achieved by carefully controlling (1) the amount of sulfur in the medium at the time of sulfur deprivation and (2) the culture density at the start of the H_2-production phase.

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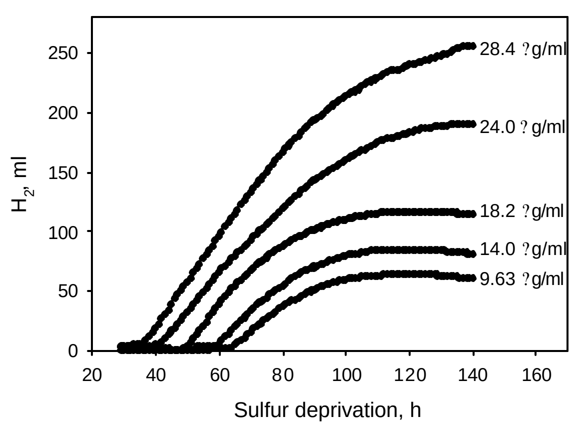

Fugure 3. Influence of culture density on H_2 production in unsynchronized cultures of *C. reinhardtii.*

References

[1] Melis A, Zhang L, Forestier M, Ghirardi ML, Seibert M. 2000. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol* 122:127-136.
[2] Ghirardi ML, Zhang L, Lee JW, Flynn T, Seibert M, Greenbaum E, Melis A. 2000. Microalgae: a green source of renewable H_2. *Trends Biotechnol* 18:506-511.
[3] Ghirardi ML, Kosourov S, Tsygankov A, Seibert M. 2000. Two-phase photobiological algal H_2-production system. *Proceedings of the 2000 DOE Hydrogen Program Review* NREL/CP-570-28890.